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(54) Title: COMBINED THERAPY AGAINST TUMORS COMPRISING SUBSTITUTED ACRYLOYL DISTAMYCIN DERIVATIVES AND PROTEIN KINASE (SERINE/THREONINE KINASE) INHIBITORS

(57) Abstract: The present invention provides the combined use of acryloyl distamycin derivatives, in particular α-bromo- and α-chloro-acryloyl distamycin derivatives of formula (I), as set forth in the specification, and a protein kinase (serine/threonine and tyrosine kinases) inhibitor, in the treatment of tumors. Also provided is the use of the said combinations in the treatment or prevention of metastasis or in the treatment of tumors by inhibition of angiogenesis.

COMBINED THERAPY AGAINST TUMORS COMPRISING SUBSTITUTED ACRYLOYL DISTAMYCIN DERIVATIVES AND PROTEIN KINASE (SERINE/THREONINE KINASE) INHIBITORS

The present invention relates to the field of cancer treatment and provides an antitumor composition comprising a substituted acryloyl distamycin derivative, more particularly an α-bromo- or α-chloro-acryloyl distamycin derivative, and a protein kinase (serine/threonine and tyrosine kinases) inhibitor, having a synergistic antineoplastic effect.

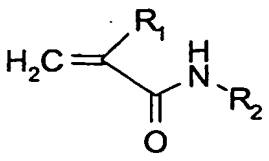
Distamycin A and analogues thereof, hereinafter referred to as distamycin and distamycin-like derivatives, are known in the art as cytotoxic agents useful in antitumor therapy.

Distamycin A is an antibiotic substance with antiviral and antiprotozoal activity, having a polypyrrole framework [*Nature* 203: 1064 (1964); *J. Med. Chem.* 32: 774-778 (1989)].

The international patent applications WO 90/11277, WO 98/04524, WO 98/21202, WO 99/50265, WO 99/50266 and WO 01/40181, all in the name of the applicant itself and herewith incorporated by reference, disclose acryloyl distamycin derivatives wherein the amidino moiety of distamycin is optionally replaced by nitrogen-containing ending groups such as, for instance, cyanamidino, N-methylamidino, guanidino, carbamoyl, amidoxime, cyano and the like, and/or wherein the polypyrrole framework of distamycin, or part of it, is replaced by varying carbocyclic or heterocyclic moieties.

The present invention provides, in a first aspect, a pharmaceutical composition for use in antineoplastic therapy in mammals, including humans, comprising a pharmaceutically acceptable carrier or excipient;

- an acryloyl distamycin derivative of formula (I):



(I)

wherein:

R_1 is a bromine or chlorine atom;

R_2 is a distamycin or distamycin-like framework; or a pharmaceutically acceptable salt thereof; and

- a protein kinase inhibitor.

The present invention includes, within its scope, the pharmaceutical compositions comprising any of the possible isomers covered by the compounds of formula (I), both considered separately or in admixture, as well as the metabolites and the pharmaceutically acceptable bio-precursors (otherwise known as pro-drugs) of the compounds of formula (I).

In the present description, unless otherwise specified, with the term distamycin or distamycin-like framework R_2 we intend any moiety structurally closely related to distamycin itself, for instance by optionally replacing the ending amidino moiety of distamycin and/or its polypyrrole framework, or part of it, for instance as set forth below.

Protein kinases, hereinafter shrortly referred to as PKs, are a large family of homologous proteins [see, for a reference, *J. Clin. Invest.* 105: 3 (2000); *Cancer Chemotherapy and Biological Response Modifiers, Annual 19* Chapter 11, 236 (2001)].

PKs, as components of signal transduction pathways, play a central role in diverse biological processes such as control of cell growth, metabolism, differentiation, and apoptosis. The development of selective PK inhibitors that can block or modulate diseases with defects in these signaling pathways, has been considered as a promising approach for the development of new anticancer drugs. A selection of these agents is shown in Table 1.

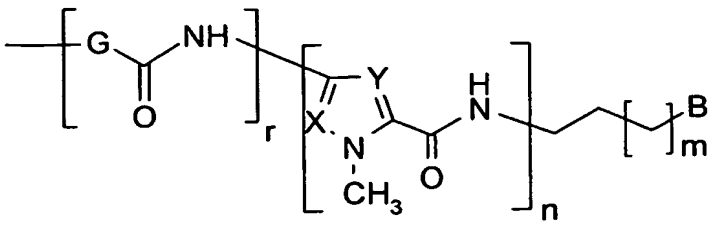
Table 1: Low Molecular weight ATP-competitive protein kinase inhibitors in clinical development

Target Kinase	Name
Bcr-Abl	STI571 (Gleevec; Imatinib)
EGF-R	ZD-1839 (Iressa) OSI-774 (Tarceva) PKI 166 EKB-569 GW572016
PKC/Trk	CEP 2563
PKC	UCN-01 GCP 41251 (STI 412) Safingol Perifosine
VEGF-R	SU 5416 (Semaxanib) CGP 79787 CP-564959 ZD 6474 ZD 2171 SU-11248
CDKs	Flavopiridol CI-202

The compositions of the invention may be thus comprised by the aforementioned acryloyl distamycin derivative of formula (I) and a protein kinase inhibitor, as listed in table 1.

According to a preferred embodiment of the invention, the PKs inhibitor is selected from STI571 (Gleevec; Imatinib - inhibitor of Bcr-Abl tyrosine kinase), ZD-1839 (Iressa – inhibitor of epidermal growth factor receptor 1 tyrosine kinase), OSI-774 (Tarceva - inhibitor of epidermal growth factor receptor 1 tyrosine kinase) and SU 5416 (Semaxanib - tyrosine kinase inhibitor that inhibits three distinct growth factor receptor targets).

According to another preferred embodiment of the invention, herewith provided are the above pharmaceutical compositions wherein, within the acryloyl distamycin derivative of formula (I), R_1 has the above reported meanings and R_2 is a group of formula (II) below:



(II)

wherein

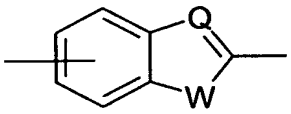
m is an integer from 0 to 2;

n is an integer from 2 to 5;

r is 0 or 1;

X and Y are, the same or different and independently for each heterocyclic ring, a nitrogen atom or a CH group;

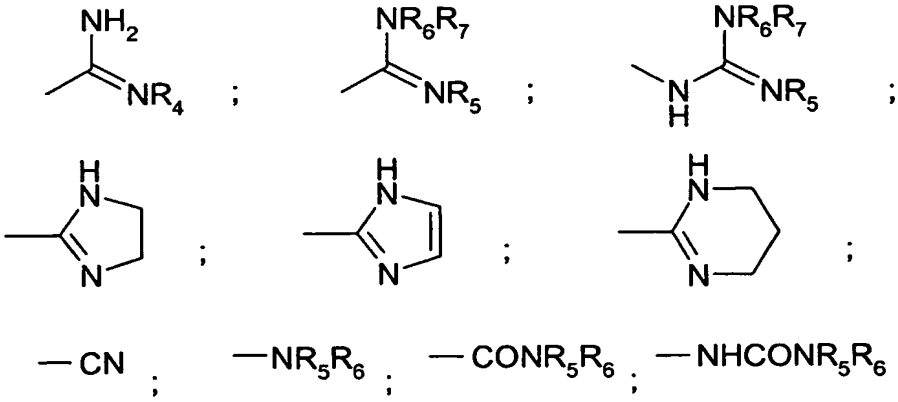
G is phenylene, a 5 or 6 membered saturated or unsaturated heterocyclic ring with from 1 to 3 heteroatoms selected among N, O or S, or it is a group of formula (III) below:



(III)

wherein Q is a nitrogen atom or a CH group and W is an oxygen or sulfur atom or it is a group NR_3 wherein R_3 is hydrogen or C_1-C_4 alkyl;

B is selected from the group consisting of



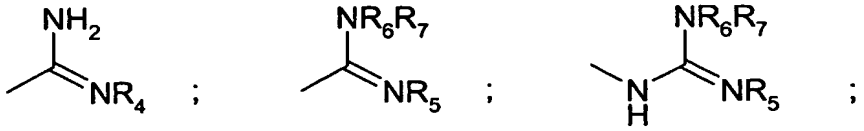
wherein R_4 is cyano, amino, hydroxy or C_1-C_4 alkoxy; R_5, R_6 and R_7, the same or different, are hydrogen or C_1-C_4 alkyl.

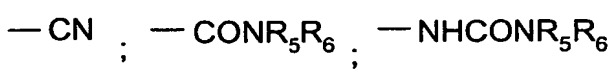
In the present description, unless otherwise specified, with the term C_1-C_4 alkyl or alkoxy group we intend a straight or branched group selected from methyl, ethyl, n-

propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, isobutoxy, sec-butoxy or tert-butoxy.

Preferably, the pharmaceutical compositions of the invention comprise the above acryloyl distamycin derivative of formula (I) wherein R_1 is bromine or chlorine; R_2 is the above group of formula (II) wherein r is 0, m is 0 or 1, n is 4 and B has the above reported meanings.

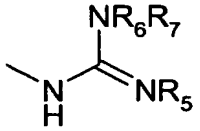
Still more preferred, within this class, are the pharmaceutical compositions comprising the compounds of formula (I) wherein R_1 is bromine or chlorine; R_2 is the above group of formula (II) wherein r is 0, m is 0 or 1, n is 4, X and Y are both CH groups and B is selected from:





wherein R_4 is cyano or hydroxy and R_5, R_6 and R_7, the same or different, are hydrogen or C_1-C_4 alkyl.

Even more preferred compositions of the invention are those comprising a compound of formula (I) wherein R_1 is bromine, R_2 is the above group of formula (II) wherein r and m are 0, n is 4, X and Y are CH, B is a group of formula:



wherein R_5, R_6 and R_7 are hydrogen atoms, optionally in the form of a pharmaceutically acceptable salt thereof.

Pharmaceutically acceptable salts of the compounds of formula (I) are those with pharmaceutically acceptable inorganic or organic acids such as, for instance, hydrochloric, hydrobromic, sulfuric, nitric, acetic, propionic, succinic, malonic, citric, tartaric, methanesulfonic, p-toluenesulfonic acid and the like.

Examples of preferred acryloyl distamycin derivatives of formula (I), within the

compositions object of the invention, for instance in the form of pharmaceutically acceptable salts, preferably with hydrochloric acid, are:

1. N-[5-[[[5-[[[2-[(aminoiminomethyl)amino]ethyl]amino]carbonyl]-1-methyl-1H-pyrrol-3-yl]amino]carbonyl]-1-methyl-1H-pyrrol-3-yl]-4-[[[4-[(2-bromo-1-oxo-2-propenyl)amino]-1-methyl-1H-pyrrol-2-yl[carbonyl]amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride (Brostallicin);
2. N-(5-{[(5-{[(5-{[(2-{[amino(imino)methyl]amino}propyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride;
3. N-(5-{[(5-{[(5-{[(3-amino-3-iminopropyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride;
4. N-(5-{[(5-{[(5-{[(3-amino-3-iminopropyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-imidazole-2-carboxamide hydrochloride;
5. N-(5-{[(5-{[(5-{[(3-amino-3-iminopropyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-3-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrazole-5-carboxamide hydrochloride;
6. N-(5-{[(5-{[(5-{[(3-amino-3-oxopropyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-3-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrazole-5-carboxamide;
7. N-(5-{[(5-{[(5-{[(2-{[amino(imino)methyl]amino}ethyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-chloroacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride;

8. N-(5-{[(5-{[(3-{[amino(imino)methyl]amino}propyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride;
9. N-(5-{[(5-{[(3-amino-3-iminopropyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride; and
10. N-{5-[({5-[({5-[({3-[(aminocarbonyl)amino]propyl}amino)carbonyl]-1-methyl-1H-pyrrol-3-yl}amino)carbonyl]-1-methyl-1H-pyrrol-3-yl}amino)carbonyl]-1-methyl-1H-pyrrol-3-yl}-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide.

The above compounds of formula (I), either specifically identified as such or by means of the general formula, are known or easily prepared according to known methods as reported, for instance, in the aforementioned international patent applications WO 90/11277, WO 98/04524, WO 98/21202, WO 99/50265 and WO 99/50266 as well as in WO 01/40181.

The present invention further provides a product, otherwise referred to as kit of parts, comprising an acryloyl distamycin derivative of formula (I), as defined above, and a PK inhibitor, as a combined preparation for simultaneous, separate or sequential use in antitumor therapy.

A further aspect of the present invention is to provide a method of treating a mammal, including humans, suffering from a neoplastic disease state, which method comprises administering to said mammal the above acryloyl distamycin derivative of formula (I) and a PK inhibitor, in amounts effective to produce a synergistic antineoplastic effect.

The present invention also provides a method for lowering the side effects caused by antineoplastic therapy with an antineoplastic agent in a mammal in need thereof, including humans, the method comprising administering to said mammal a combined preparation comprising a PK inhibitor and an acryloyl distamycin derivative of formula (I), as defined above, in amounts effective to produce a synergistic antineoplastic effect.

By the term "synergistic antineoplastic effect", as used herein, it is meant the inhibition of the growth tumor, preferably the complete regression of the tumor, by administering an effective amount of the combination comprising an acryloyl distamycin derivative of formula (I) and a PK inhibitor to mammals, including humans.

By the term "administered " or "administering", as used herein, it is meant parenteral and/or oral administration; the term "parenteral" means intravenous, subcutaneous and intramuscular administration.

In the method of the present invention, the acryloyl distamycin derivative may be administered simultaneously with the PK inhibitor or, alternatively, both compounds may be administered sequentially in either order.

In this respect, it will be appreciated that the actual preferred method and order of administration will vary according to, inter alia, the particular formulation of the acryloyl distamycin of formula (I) being used, the particular formulation of the PK inhibitor being used, the particular tumor model being treated as well as the particular host being treated.

To administer the acryloyl distamycin derivative of formula (I), according to the method of the invention, the course of therapy generally employed comprises doses varying from about 0.05 to about 100 mg/m^2 of body surface area and, more preferably, from about 0.1 to about 50 mg/m^2 of body surface area.

For the administration of the PK inhibitor, according to the method of the invention, the course of therapy generally employed may be as follows.

For the administration of STI571 (Imatinib), doses varying from about 5 mg/day to about 5000 mg/day and, more preferably, from about 30 to about 1000 mg/day.

For the administration of ZD 1839 (Iressa) doses varying from about 5 mg/day to about 10000 mg/day and, more preferably, from about 50 to about 1000 mg/day.

For the administration of OSI-774 (Tarceva) doses varying from about 5 mg/day to about 10000 mg/day and, more preferably, from about 50 to about 1000 mg/day.

For the administration of SU 5416 (Semaxanib) doses varying from about 1 mg/m^2 to about 1000 mg/m^2 of body surface area and, more preferably, from about 10 to about 500 mg/m^2 of body surface area.

The antineoplastic therapy of the present invention is particularly suitable for treating breast, ovary, lung, colon, kidney, stomach, pancreas, liver, melanoma, leukemia and brain tumors in mammals, including humans.

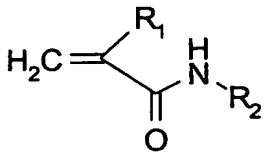
In a further aspect, the present invention is directed to a pharmaceutical composition comprising an effective amount of an acryloyl distamycin derivative of formula (I), as defined above, and a PK inhibitor, in the preparation of a medicament for use in the prevention or treatment of metastasis or in the treatment of tumors by inhibition of angiogenesis.

As the effect of an acryloyl distamycin derivative of formula (I) and a PK inhibitor is significantly increased without a parallel increase of toxicity, the combined therapy of the present invention enhances the antitumoral effects of the acryloyl distamycin derivative and of the PK inhibitor and, hence, provides the most effective and least toxic treatment for tumors.

CLAIMS

1. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or excipient and, as active ingredient,

- an acryloyl distamycin derivative of formula (I):



(I)

wherein:

R_1 is a bromine or chlorine atom;

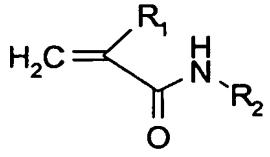
R_2 is a distamycin or distamycin-like framework; or a pharmaceutically acceptable salt thereof; and

- a protein kinase inhibitor.

2. A pharmaceutical composition according to claim 1 wherein the protein kinase inhibitor is selected from the group consisting of STI571, ZD-1839, OSI-774, PKI 166, EKB-569, GW572016, CEP 2563, UCN-01, GCP 41251 (STI 412), Safingol, Perifosine, SU 5416, CGP 79787, CP-564959, ZD 6474, ZD 2171, SU-11248, Flavopiridol, and CI-202.

3. A pharmaceutical composition according to claim 2 wherein the protein kinase inhibitor is selected from the group consisting of STI571, ZD-1839, OSI-774 and SU 5416.

4. A pharmaceutical composition according to claim 1 comprising an acryloyl distamycin derivative of formula (I)

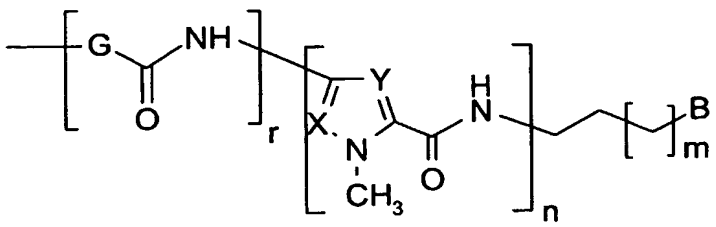


(I)

wherein:

R_1 is a bromine or chlorine atom;

R_2 is a group of formula (II)



(II)

wherein

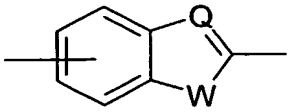
m is an integer from 0 to 2;

n is an integer from 2 to 5;

r is 0 or 1;

X and Y are, the same or different and independently for each heterocyclic ring, a nitrogen atom or a CH group;

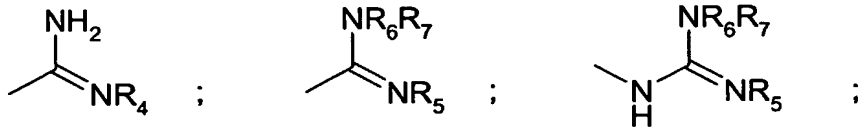
G is phenylene, a 5 or 6 membered saturated or unsaturated heterocyclic ring with from 1 to 3 heteroatoms selected among N, O or S, or it is a group of formula (III) below:

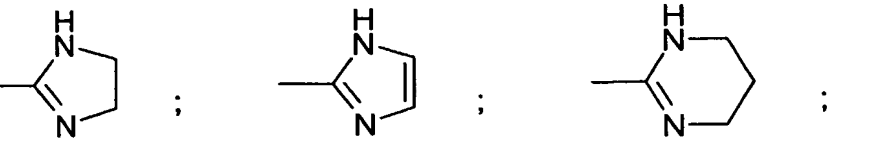


(III)

wherein Q is a nitrogen atom or a CH group and W is an oxygen or sulfur atom or it is a group NR_3 wherein R_3 is hydrogen or C_1-C_4 alkyl;

B is selected from the group consisting of

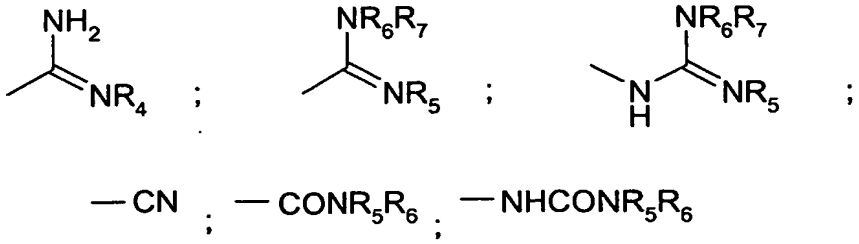




wherein R_4 is cyano, amino, hydroxy or C_1-C_4 alkoxy; R_5, R_6 and R_7, the same or different, are hydrogen or C_1-C_4 alkyl.

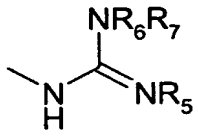
5. A pharmaceutical composition according to claim 4 comprising an acryloyl

distamycin derivative of formula (I) wherein R_1 and R_2 are as defined in claim 4, r is 0, m is 0 or 1, n is 4, X and Y are both CH groups and B is selected from:



wherein R_4 is cyano or hydroxy and R_5, R_6 and R_7, the same or different, are hydrogen or C_1-C_4 alkyl.

6. A pharmaceutical composition according to claim 5 comprising an acryloyl distamycin derivative of formula (I) wherein R_1 is bromine, R_2 is a group of formula (II) wherein r and m are 0, n is 4, X and Y are CH, B is a group of formula



wherein R_5, R_6 and R_7 are hydrogen atoms, optionally in the form of a pharmaceutically acceptable salt.

7. A pharmaceutical composition according to claim 1 comprising an acryloyl distamycin derivative, optionally in the form of a pharmaceutically acceptable salt, selected from the group consisting of:

1. N-[5-[[[5-[[[2-[(aminoiminomethyl)amino]ethyl]amino]carbonyl]-1-methyl-1H-pyrrol-3-yl]amino]carbonyl]-1-methyl-1H-pyrrol-3-yl]-4-[[[4-[(2-bromo-1-oxo-2-propenyl)amino]-1-methyl-1H-pyrrol-2-yl[carbonyl]amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride (Brostallicin);
2. N-(5-{[(5-{[(5-{[(2-{[amino(imino)methyl]amino}propyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride;
3. N-(5-{[(5-{[(5-{[(3-amino-3-iminopropyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-

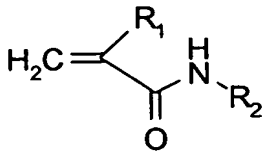
pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride;

4. N-(5-{[(5-{[(5-{[(3-amino-3-iminopropyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-imidazole-2-carboxamide hydrochloride;
5. N-(5-{[(5-{[(5-{[(3-amino-3-iminopropyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-3-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrazole-5-carboxamide hydrochloride;
6. N-(5-{[(5-{[(5-{[(3-amino-3-oxopropyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-3-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrazole-5-carboxamide;
7. N-(5-{[(5-{[(5-{[(2-{[amino(imino)methyl]amino}ethyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-chloroacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride;
8. N-(5-{[(5-{[(3-{[amino(imino)methyl]amino}propyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride;
9. N-(5-{[(5-{[(3-amino-3-iminopropyl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)amino]carbonyl}-1-methyl-1H-pyrrol-3-yl)-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride; and
10. N-{5-[({5-[({5-[({3-[(aminocarbonyl)amino]propyl}amino)carbonyl]-1-methyl-1H-pyrrol-3-yl}amino)carbonyl]-1-methyl-1H-pyrrol-3-yl}amino)carbonyl]-1-methyl-1H-pyrrol-3-yl}-4-[(2-bromoacryloyl)amino]-1-methyl-1H-pyrrole-2-carboxamide.

8. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or excipient and, as active ingredient,

- N-[5-[[[5-[[[2-[(aminoiminomethyl)amino]ethyl]amino]carbonyl]-1-methyl-1H-pyrrol-3-yl]amino]carbonyl]-1-methyl-1H-pyrrol-3-yl]-4-[[[4-[(2-bromo-1-oxo-2-propenyl)amino]-1-methyl-1H-pyrrol-2-yl[carbonyl]amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride (Brostallicin); and
- a protein kinase inhibitor selected from the group consisting of STI571, ZD-1839, OSI-774, and SU 5416.

9. Products comprising an acryloyl distamycin derivative of formula (I):



(I)

wherein:

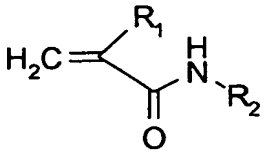
R_1 is a bromine or chlorine atom;

R_2 is a distamycin or distamycin-like framework; or a pharmaceutically acceptable salt thereof; and a protein kinase inhibitor, as a combined preparation for simultaneous, separate or sequential use in the treatment of tumors.

10. Products according to claim 9 wherein the protein kinase inhibitor is selected from the group consisting of STI571, ZD-1839, OSI-774, PKI 166, EKB-569, GW572016, CEP 2563, UCN-01, GCP 41251 (STI 412), Safingol, Perifosine, SU 5416, CGP 79787, CP-564959, ZD 6474, ZD 2171, SU-11248, Flavopiridol, and CI-202.

11. Products according to claim 10 wherein the protein kinase inhibitor is selected from the group consisting of STI571, ZD-1839, OSI-774 and SU 5416.

12. Products according to claim 9 comprising an acryloyl distamycin derivative of formula (I)

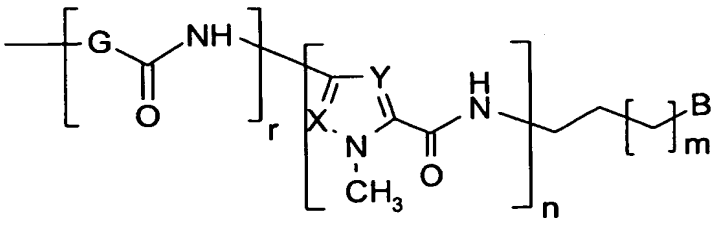


(I)

wherein:

R_1 is a bromine or chlorine atom;

R_2 is a group of formula (II)



(II)

wherein

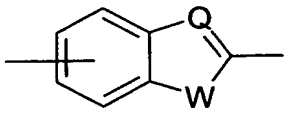
m is an integer from 0 to 2;

n is an integer from 2 to 5;

r is 0 or 1;

X and Y are, the same or different and independently for each heterocyclic ring, a nitrogen atom or a CH group;

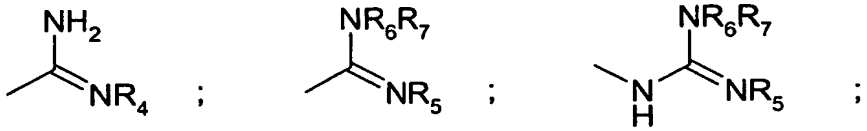
G is phenylene, a 5 or 6 membered saturated or unsaturated heterocyclic ring with from 1 to 3 heteroatoms selected among N, O or S, or it is a group of formula (III) below:

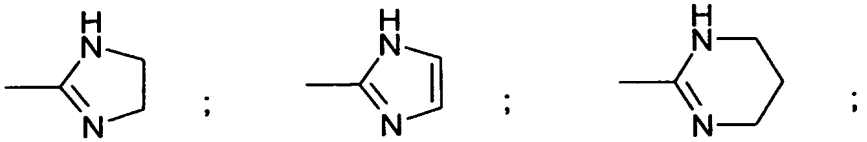


(III)

wherein Q is a nitrogen atom or a CH group and W is an oxygen or sulfur atom or it is a group NR_3 wherein R_3 is hydrogen or C_1-C_4 alkyl;

B is selected from the group consisting of

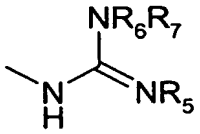




$-CN$; $-NR_5R_6$; $-CONR_5R_6$; $-NHCONR_5R_6$

wherein R_4 is cyano, amino, hydroxy or C_1-C_4 alkoxy; R_5, R_6 and R_7, the same or different, are hydrogen or C_1-C_4 alkyl.

13. Products according to claim 9 comprising an acryloyl distamycin derivative of formula (I) wherein R_1 is bromine, R_2 is a group of formula (II) wherein r and m are 0, n is 4, X and Y are CH, B is a group of formula



wherein R_5, R_6 and R_7 are hydrogen atoms, optionally in the form of a pharmaceutically acceptable salt.

14. Products according to claim 9 wherein the acryloyl distamycin derivative is selected from the group as defined in claim 7.

15. Products comprising the acryloyl distamycin derivative N-[5-[[[5-[[[2-[(aminoiminomethyl)amino]ethyl]amino]carbonyl]-1-methyl-1H-pyrrol-3-yl]amino]carbonyl]-1-methyl-1H-pyrrol-3-yl]-4-[[[4-[(2-bromo-1-oxo-2-propenyl)amino]-1-methyl-1H-pyrrol-2-yl[carbonyl]amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride (Brostallicin), and a protein kinase inhibitor selected from the group consisting of STI571, ZD-1839, OSI-774, and SU 5416; as a combined preparation for simultaneous, separate or sequential use in the treatment of tumors.

16. Use of an acryloyl distamycin derivative of formula (I), as defined in claim 1, in the preparation of a medicament to be used in combination therapy with a protein kinase inhibitor, in the treatment of tumors.

17. Use according to claim 16 wherein the medicament further comprises the said protein kinase inhibitor.

18. Use according to claims 16 or 17 wherein the protein kinase inhibitor is as defined in claim 2.

19. Use according to claims 16 or 17 wherein the acryloyl distamycin derivative is selected from the group as defined in claim 7.

20. Use of the acryloyl distamycin derivative N-[5-[[[5-[[[2-[(aminoiminomethyl)amino]ethyl]amino]carbonyl]-1-methyl-1H-pyrrol-3-yl]amino]carbonyl]-1-methyl-1H-pyrrol-3-yl]-4-[[[4-[(2-bromo-1-oxo-2-propenyl)amino]-1-methyl-1H-pyrrol-2-yl[carbonyl]amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride (Brostallicin), in the preparation of a medicament to be used in combination therapy with a protein kinase inhibitor selected from the group consisting of STI571, ZD-1839, OSI-774, and SU 5416, in the treatment of tumors.

21. Use according to any one of claims from 16 to 20 wherein the tumor is selected from breast, ovary, lung, colon, kidney, stomach, pancreas, liver, melanoma, leukemia and brain tumors.

22. Use of an acryloyl distamycin derivative of formula (I), as defined in claim 1, in the preparation of a medicament to be used in combination therapy with a protein kinase inhibitor, in the prevention or treatment of metastasis or in the treatment of tumors by inhibition of angiogenesis.

23. Use according to claim 22 wherein the medicament further comprises the said protein kinase inhibitor.

24. A method of treating a mammal, including humans, suffering from a neoplastic disease state, which method comprises administering to said mammal the acryloyl distamycin derivative of formula (I), as defined in claim 1, and a protein kinase inhibitor, in amounts effective to produce a synergistic antineoplastic effect.

25. A method according to claim 24 wherein the acryloyl distamycin derivative is N-[5-[[[5-[[[2-[(aminoiminomethyl)amino]ethyl]amino]carbonyl]-1-methyl-1H-pyrrol-3-yl]amino]carbonyl]-1-methyl-1H-pyrrol-3-yl]-4-[[[4-[(2-bromo-1-oxo-2-

propenyl)amino]-1-methyl-1H-pyrrol-2-yl[carbonyl]amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride (Brostallicin), and the protein kinase inhibitor is selected from the group consisting of STI571, ZD-1839, OSI-774, and SU 5416.

26. A method for lowering the side effects caused by antineoplastic therapy with an antineoplastic agent, in a mammal in need thereof including humans, the method comprising administering to said mammal a combined preparation comprising a protein kinase inhibitor and an acryloyl distamycin derivative of formula (I), as defined in claim 1, in amounts effective to produce a synergistic antineoplastic effect.

27. A method according to claim 26 wherein the acryloyl distamycin derivative is N-[5-[[[5-[[[2-[(aminoiminomethyl)amino]ethyl]amino]carbonyl]-1-methyl-1H-pyrrol-3-yl]amino]carbonyl]-1-methyl-1H-pyrrol-3-yl]-4-[[[4-[(2-bromo-1-oxo-2-propenyl)amino]-1-methyl-1H-pyrrol-2-yl[carbonyl]amino]-1-methyl-1H-pyrrole-2-carboxamide hydrochloride (Brostallicin), and the protein kinase inhibitor is selected from the group consisting of STI571, ZD-1839, OSI-774, and SU 5416.

INTERNATIONAL SEARCH REPORT

PCT/EP[redacted]/13092

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K45/06 A61K31/40 A61K31/415 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 01 97789 A (PHARMACIA & UPJOHN) 27 December 2001 (2001-12-27) claims 1,4-7,13,17,18,20,21	1,4-9, 12-16, 19-22, 24-27
A	WO 01 97790 A (PHARMACIA & UPJHN) 27 December 2001 (2001-12-27) claims 1,3-7,9-11,14-16,18-20	1,4-9, 12-16, 19-22, 24-26

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 March 2003	01/04/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Peeters, J

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	M.CIOMEI E.A.: "Decreased tyrosine phosphorylation in tumour cells resistant to FCE 24517 (tallimustine)" BRITISH JOURNAL OF CANCER, vol. 72, no. 6, 1995, pages 1504-1508, XP008015252 page 1504, column 1 page 1506 page 1507, column 1 ---	1,9,16, 17,21-24
A	S.MARCHINI E.A.: "Alpha-bromoacryloyl derivative of distamycin A (PNU 151807):a new non-covalent minor groove DNA binder with antineoplastic activity" BRITISH JOURNAL OF CANCER, vol. 80, no. 7, 1999, pages 991-997, XP008015251 page 991 -page 992 -----	1,4-7,9, 12-14, 16,17, 21-23

INTERNATIONAL SEARCH REPORT

PCT/02/13092

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: –
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [X] Claims Nos.: –
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 24 and 25 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

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Continuation of Box I.2

Present claims 1-6,9-13,16-18,21-24,26 relate to an extremely large number of possible compounds/products/methods. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds/products/methods claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely claims 7,8,14,15,19,20,25,27, with due regard to the general idea underlying the present application.

Present claims 1,4-7,9,12-14,16,17,19,21-24,26 relate to a product/compound/method defined by reference to a desirable characteristic or property, namely "Protein kinase inhibitor"

The claims cover all products/compounds/methods having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such products/compounds/methods. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product/compound/method by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely claims 2,3,8,10,11,15,18,20,25,27, with due regard to the general idea underlying the present application.

The applicant's attention is drawn to the fact that claims, or parts of

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information [redacted] patent family members

PCT/EP [redacted]3092

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 0197789	A	27-12-2001	AU	7846301 A	02-01-2002
			WO	0197789 A2	27-12-2001
			EP	1292290 A2	19-03-2003
			NO	20026078 A	18-12-2002
WO 0197790	A	27-12-2001	AU	8186701 A	02-01-2002
			WO	0197790 A2	27-12-2001
			NO	20026077 A	18-12-2002